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Cellular and Ionic Mechanism for Drug-Induced Long QT Syndrome and Effectiveness of Verapamil

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OBJECTIVES We examined the cellular and ionic mechanism for QT prolongation and subsequent Torsade de Pointes (TdP) and the effect of verapamil under conditions mimicking *KCNQ1* (I_{Ks} gene) defect linked to acquired long QT syndrome (LQTS).

BACKGROUND Agents with an I_{Kr}-blocking effect often induce marked QT prolongation in patients with acquired LQTS. Previous reports demonstrated a relationship between subclinical mutations in cardiac K^+ channel genes and a risk of drug-induced TdP.

METHODS Transmembrane action potentials from epicardial (EPI), midmyocardial (M), and endocardial (ENDO) cells were simultaneously recorded, together with a transmural electrocardiogram, at a basic cycle length of 2,000 ms in arterially perfused feline left ventricular preparations.

RESULTS The I_{Kr} block (E-4031: 1 μmol/l) under control conditions (n = 5) prolonged the QT interval but neither increased transmural dispersion of repolarization (TDR) nor induced arrhythmias. However, the I_{Kr} blocker under conditions with I_{Ks} suppression by chromanol 293B 10 μmol/l mimicking the *KCNQ1* defect (n = 10) preferentially prolonged action potential duration (APD) in EPI rather than M or ENDO, thereby dramatically increasing the QT interval and TDR. Spontaneous or epinephrine-induced early afterdepolarizations (EADs) were observed in EPI, and subsequent TdP occurred only under both I_{Ks} and I_{Kr} suppression. Verapamil (0.1 to 5.0 μmol/l) dose-dependently abbreviated APD in EPI more than in M and ENDO, thereby significantly decreasing the QT interval, TDR, and suppressing EADs and TdP.

CONCLUSIONS Subclinical I_{Ks} dysfunction could be a risk of drug-induced TdP. Verapamil is effective in decreasing the QT interval and TDR and in suppressing EADs, thus preventing TdP in the model of acquired LQTS. (J Am Coll Cardiol 2005;45:300–7) © 2005 by the American College of Cardiology Foundation

The long QT syndrome (LQTS) is characterized by a prolongation of ventricular repolarization and recurrent episodes of atypical polymorphic ventricular tachycardia known as Torsade de Pointes (TdP) leading to sudden cardiac death (1–3). The molecular basis of congenital LQTS is attributed to defects in several ion channel genes encoding delayed rectifier K^+ or Na^+ currents. On the other hand, agents that block rapidly activating delayed rectifier potassium current (I_{Kr}) often induce marked QT prolongation with an inverted T wave in patients with acquired LQTS. Recent studies indicate that some cases of drug-induced LQTS can be associated with silent mutations and common polymorphism in genes responsible for the congenital LQTS (4), such as *KCNQ1* encoding slowly activating delayed rectifier potassium currents (I_{Ks}) (5–7). However, it remains unclear why subclinical I_{Ks} dysfunction is a risk of drug-induced LQTS.

Both early afterdepolarization (EAD)-induced triggered activity and increased dispersion of repolarization have been suggested as important in the genesis of ventricular arrhythmias in congenital and acquired LQTS. Moreover, verapamil, an L-type Ca^{2+} channel blocker, suppressed EADs and TdP in patients with LQTS (8,9). In the present study, we hypothesized that: 1) addition of I_{Kr} block to I_{Ks} dysfunction markedly prolongs action potential duration (APD) and induces TdP by producing EADs and/or increases transmural dispersion of repolarization (TDR); and 2) verapamil suppresses TdP by preventing EADs and decreasing TDR. In arterially perfused feline left ventricular wedge preparations, we demonstrated that subclinical I_{Ks} dysfunction, mimicking *KCNQ1* defect, could be a risk of drug-induced TdP, and verapamil successfully suppressed TdP in the model of acquired LQTS.

METHODS

Arterially perfused wedge preparations and electrophysiologic recordings. All animal care procedures were in accordance with the position of the American Heart Association research animal use (November 11, 1984). The

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Abbreviations and Acronyms

APD_{90} = action potential duration measured at 90% repolarization
BCL = basic cycle length
EAD = early afterdepolarization
I_K = delayed rectifier potassium current
I_{Kr} = rapidly activating delayed rectifier potassium current
I_{Ks} = slowly activating delayed rectifier potassium current
LQTS = long QT syndrome
TdP = Torsade de Pointes
TDR = transmural dispersion of repolarization

methods used for isolation, perfusion, and recording of transmembrane activity from the arterially perfused feline left ventricle have been detailed in a previous study (10) and are similar to methods reported using canine or rabbit wedge preparations (11–15). Briefly, a transmural wedge was dissected from the anterior wall of the left ventricle, cannulated via the left descending coronary artery (or the first branch of the left circumflex), and placed in a small tissue bath arterially perfused with Tyrode's solution. The temperature was maintained at 37 ± 1°C and perfusion pressure maintained between 40 and 60 mm Hg. Ventricular wedges were stimulated with bipolar electrodes applied to the endocardial surface. We recorded a transmural electrocardiogram (ECG) (epicardial, positive pole) using Ag-AgCl electrodes, and transmembrane action potentials (APs) simultaneously from the epicardium, midmyocardium (M), and endomyocardium using three separate intracellular floating microelectrodes. The epicardial and endocardial APs were recorded from the epicardial and endocardial surfaces, respectively, at positions approximating the transmural axis of the ECG. The M-cell's AP was recorded from the transmural surface, mainly at the subendocardium, along the same axis.

An I_{Kr} blocker, E-4031 1 μmol/l, was used in control condition (n = 5) or under condition with I_{Ks} suppression by chromanol 293B 10 μmol/l, mimicking *KCNQ1* defect (n = 10). The effects of an L-type Ca^{2+} channel blocker, verapamil, were evaluated at 0.1, 1, 2.5, and 5 μmol/l under the I_{Ks} and I_{Kr} suppression (acquired LQTS condition). Epinephrine 0.5 μmol/l was used to mimic increased sympathetic activity in the absence and presence of verapamil under the acquired LQTS condition. The spontaneous or epinephrine-induced EADs and subsequent TdP were evaluated under each set of conditions.

Data using E-4031, 293B, 293B + E-4031, and additional verapamil on top of 293B + E-4031 were collected for a period of 30 min starting 30 min after applying the above compounds to the perfusion. The APD was measured at 90% repolarization (APD_{90}). The TDR was defined as the difference between the longest and shortest repolarization times (activation time + APD_{90}) of the APs recorded across the wall. The QT interval was defined as the time interval between the QRS onset and the point at which the line of maximal downslope of the positive T wave and the line of the maximal upslope of the negative T wave crossed the baseline.

Whole-cell patch-clamp experiments. Epicardial, M, and endocardial cells isolated from the feline left ventricle were voltage-clamped using whole-cell configuration of the patch-clamp technique (16). Patch electrodes were pulled from borosilicate glass capillaries, heat-polished, and had a tip resistance of 2.0 to 3.0 MΩ when filled with standard pipette solution containing (mmol/l): 70 potassium aspartate, 50 KCl, 10 KH_2PO_4, 1 $MgSO_4$, 3 Na_2-ATP, 0.1 Li_2-GTP, 5 EGTA, and 5 HEPES (pH adjusted to 7.2 with KOH). Membrane currents were recorded from the epicardial, M, and endocardial cells superfused at 34 to 36°C with normal Tyrode's solution containing (mmol/l): 140 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 0.5 $MgCl_2$, 0.33 NaH_2PO_4, 5.5 glucose, and 5.0 HEPES (pH adjusted to 7.4 with NaOH). In all current measurements, nisoldipine (0.4 μmol/l) was added to normal Tyrode's solution to abolish $I_{Ca,L}$. The cell membrane capacitance (C_m) was calculated for each cell by fitting the single exponential function to the decay of the capacitive transient elicited by a 5-mV step hyperpolarization applied from a holding potential of −50 mV (17).

Simulation study. Isolated epicardial, M, and endocardial cells were simulated using a Luo-Rudy dynamic cell model modified by varying the maximum conductance (density) of I_{Kr} and I_{Ks} (G_{Kr} and G_{Ks}) as described previously (18), in which the G_{Ks}/G_{Kr} in the epicardial, M, and endocardial cells were 23, 17, and 19, respectively. The transient outward potassium current (I_{to}) was incorporated into the model using the formulation of Dumaine et al. (19), in which the maximum conductance of I_{to} (G_{to}) was set to 0.5, 0.25, and 0.05 mS/μF in the epicardial, M, and endocardial cells, respectively.

Statistics. Statistical analysis of the data was performed with a Student *t* test for paired data or analysis of variance coupled with Bonferroni's test, as appropriate. Data are expressed as mean values ± SD except for those shown in the figures, which are expressed as mean ± SEM. Significance was defined as a value of $p < 0.05$.

RESULTS

The QT interval, APD, and TDR under an acquired LQTS condition with or without epinephrine. Figure 1 shows transmembrane activity recorded simultaneously from the epicardium, M, and endocardium together with a transmural ECG at a basic cycle length (BCL) of 2,000 ms. E-4031 (1 μmol/l) alone significantly, but homogenously, prolonged APD of the three regions, causing no major change in TDR (Fig. 1B). Chromanol 293B (10 μmol/l) alone did not significantly increase the QT interval, APD of the three regions, and TDR (Fig. 1C). The additional E-4031 to 293B, mimicking acquired LQTS, preferentially prolonged epicardial APD, thus dramatically increased QT

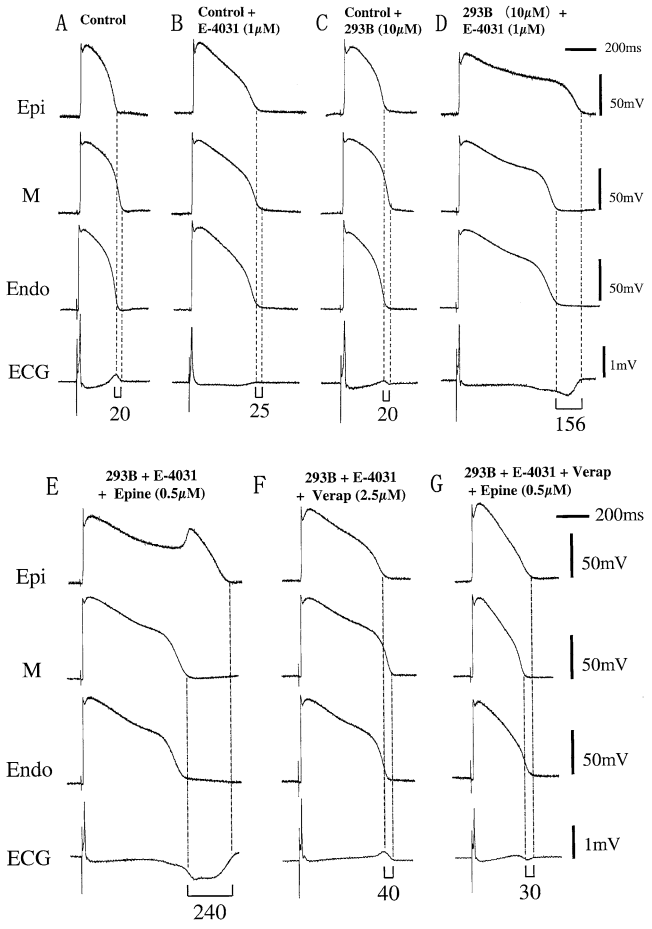

Figure 1. Transmembrane action potentials simultaneously recorded from the epicardial (Epi), midmyocardial (M), and endocardial (Endo) regions and a transmural electrocardiogram (ECG) at basic cycle length of 2,000 ms under each study condition. **(A)** Control. **(B)** E-4031 (1μmol/l). **(C)** Chromanol 293B (10μmol/l). **(D)** 293B + E-4031 (acquired long QT syndrome [LQTS] condition). **(E)** Epinephrine infusion (Epine: 0.5μmol/l) under acquired LQTS condition. **(F)** Addition of verapamil (Verap) 2.5 μmol/l under acquired LQTS condition. **(G)** Further addition of Epine in the continued presence of Verap under acquired LQTS condition. Numbers at bottom of each ECG denote transmural dispersion of repolarization (ms).

interval and TDR (Fig. 1D). Epinephrine infusion (0.5 μmol/l) further prolonged epicardial APD associated with induction of EADs, but did not prolong M or endocardial APD, resulting in further QT prolongation and increasing TDR (Fig. 1E).

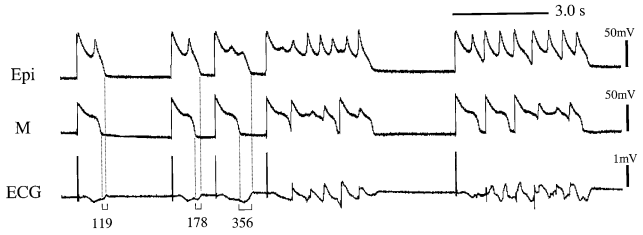

Figure 2. Spontaneous early afterdepolarization and subsequent Torsade de Pointes under the acquired long QT syndrome condition (293B 10 μmol/l + E-4031 1 μmol/l). Basic cycle length = 3,000 ms. Recordings and abbreviations as in Figure 1.

The composite data of the QT interval, APD_{90} of the epicardium, M, and endocardium, and TDR at a BCL of 2,000 ms are shown in Table 1. E-4031 under control significantly, but homogeneously, prolonged APD_{90}, resulting in neither change of TDR nor induction of arrhythmia. Chromanol 293B under control did not significantly increase APD_{90} of the three regions, resulting in no major change in QT interval and TDR. Whereas additional E-4031 to 293B markedly prolonged QT interval as evidenced by preferential prolongation of the epicardial APD_{90} compared with M and endocardial APD_{90}, thus dramatically increased TDR. Epinephrine further prolonged the epicardial APD_{90}, but shortened the M region APD_{90}, resulting in further prolongation of the QT interval and increasing TDR.

Neither E-4031 alone nor 293B alone produced any EADs or TdP. However, additional E-4031 to 293B (acquired LQTS condition) induced spontaneous EADs from the epicardium in 5 of 10 preparations, including two preparations with spontaneous TdP (Fig. 2), but not from the M or endocardium. Further epinephrine infusion (n = 8) induced EADs from the epicardium in all preparations, including four preparations with subsequent TdP, but EADs from the M region were seen in only one preparation.

Effect of verapamil on the QT interval, APD, TDR, and induction of arrhythmias under an acquired LQTS condition. Under the acquired LQTS condition, verapamil dose-dependently (0.1 to 5 μmol/l) abbreviated APD of

Table 1. Effect of I_{Kr} Block With or Without Pretreated I_{Ks} Block on the QT Interval, APD_{90}, and Transmural Dispersion of Repolarization

		APD_{90}			
	QT	Epi	M	Endo	TDR
Control (n = 5)	283 ± 15	227 ± 16	259 ± 8	246 ± 13	31 ± 10
E-4031 (1 μM) (n = 5)	446 ± 42*	373 ± 30*	408 ± 28*	374 ± 25*	34 ± 4
Control (n = 10)	279 ± 12	230 ± 16	253 ± 14	237 ± 19	24 ± 5
293B (10 μM) (n = 10)	298 ± 34	252 ± 26	275 ± 33	253 ± 16	24 ± 9
293B (10 μM) + E-4031 (1 μM) (n = 10)	793 ± 183*	723 ± 164*	596 ± 131*	545 ± 78*	175 ± 68*
293B + E-4031 + Epine (0.5 μM) (n = 8)	866 ± 251	801 ± 217	506 ± 123	525 ± 118	191 ± 75
293B + E-4031 + Verap (2.5 μM) (n = 7)	557 ± 178‡	503 ± 171‡	483 ± 135†	516 ± 154	35 ± 37‡
293B + E-4031 + Verap + Epine (n = 6)	445 ± 113‡	403 ± 117‡	399 ± 93‡	411 ± 98†	30 ± 12‡

*$p < 0.001$ vs. control, †$p < 0.05$ vs. 293B + E-4031; ‡$p < 0.01$ vs. 293B + E-4031 by analysis of variance with Bonferroni's test.

APD_{90} = action potential duration at 90% repolarization; Endo = endocardium; Epi = epicardium; Epine = epinephrine; I_{ks} = slowly activating delayed rectifier potassium current; I_{kr} = rapidly activating delayed rectifier potassium current; M = mid-myocardium; QT = QT interval; TDR = transmural dispersion of repolarization; Verap = verapamil.

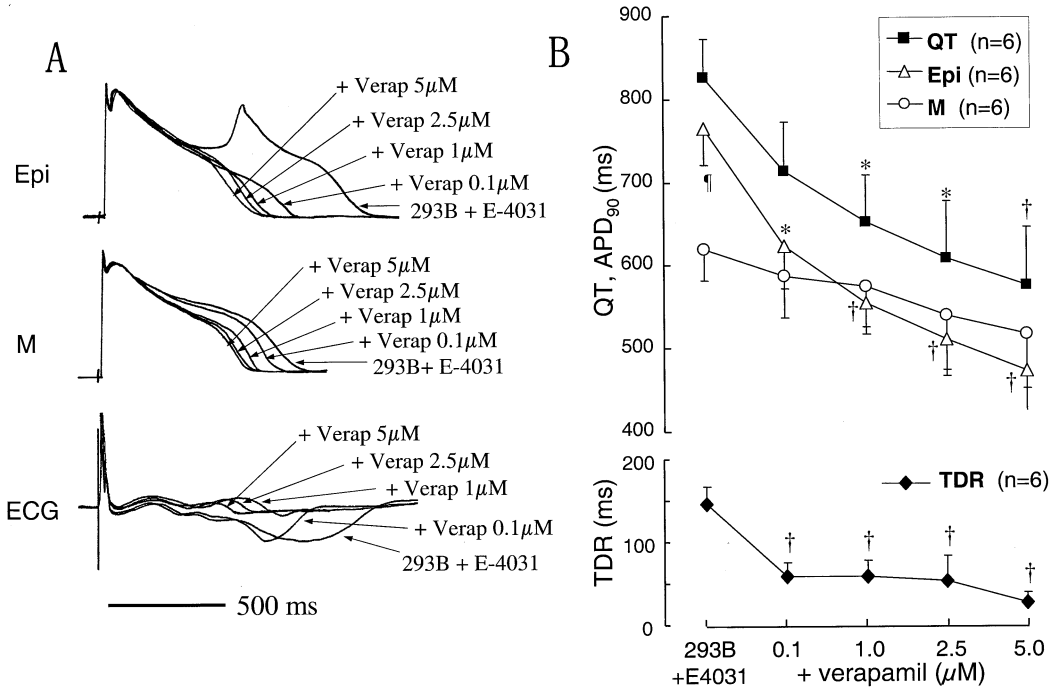

Figure 3. Dose-dependent effect of Verap (0.1 to 5 μmol/l) on transmembrane and ECG activity under acquired LQTS condition (293B 10 μmol/l + E-4031 1 μmol/l). **(A)** Superimposed action potentials recorded simultaneously from the epicardial and M regions together with a transmural ECG. **(B)** Composite data of the effect of Verap on QT interval **(solid squares)**, action potential duration measured at 90% repolarization (APD_{90}) of Epi **(open triangles)** and M **(open circles)** regions and transmural dispersion of repolarization (TDR) **(solid diamonds)**. Basic cycle length = 2,000 ms. *$p < 0.05$ vs. 293B + E-4031; †$p < 0.01$ vs. 293B + E-4031; ¶$p < 0.05$ vs. M region by analysis of variance with Bonferroni's test. Abbreviations as in Figure 1.

the epicardial and M regions as well as the QT interval (Fig. 3A). Figure 3B shows composite data of the dose-dependent effect of verapamil on the QT interval, APD_{90} of the epicardial and M regions, and TDR under the acquired LQTS condition (n = 6). A 5-μmol/l dose of verapamil under the acquired LQTS condition preferentially abbreviated the epicardial APD_{90} (761 ± 99 ms to 469 ± 95 ms; $p < 0.001$) compared with the M region APD_{90} (615 ± 83 ms to 512 ± 146 ms; p = NS), resulting in a significant decrease in TDR (146 ± 46 ms to 26 ± 28 ms; $p < 0.01$). The change in QT interval paralleled the decrease in the epicardial APD_{90}.

As shown in Figure 1F, 2.5-μmol/l verapamil preferentially abbreviated the epicardial APD_{90} rather than the M or endocardium, thus significantly abbreviated QT interval and TDR. Moreover, verapamil completely prevented the influence of epinephrine in inducing EADs and TdP as well as increasing the epicardial APD_{90}, QT interval, and TDR (Fig. 1G). The composite data of the effect of verapamil on the QT interval, APD, and TDR with or without epinephrine are shown in Table 1. Thus, verapamil totally suppressed EADs and TdP under the acquired LQTS condition with or without epinephrine.

Measurement of I_{Kr} and I_{Ks} in epicardial, M, and endocardial cells. Figure 4A represents the dose-dependent inhibition of I_{Ks} by 293B in an epicardial cell. Figure 4B illustrates the concentration-response relationships for the inhibition of I_{Ks} tail current. The data points were reasonably well described by a Hill equation with the following parameters: IC_{50} = 6.39 ± 1.17 μmol/l, n_H = 1.23 ± 0.05 (epicardial cells: n = 5); IC_{50} = 5.71 ± 1.32 μmol/l, n_H = 1.25 ± 0.12 (M cells: n = 5); IC_{50} = 5.73 ± 0.94 μmol/l, n_H = 1.07 ± 0.19 (endocardial cells: n = 5). There are no significant differences in IC_{50} and n_H values among the epicardial, M, and endocardial cells (analysis of variance with Bonferroni's test), thus indicating that I_{Ks} in these three cell types represents a similar sensitivity to inhibition by chromanol 293B.

Figure 5 represents the sensitivity of I_K to blockers of I_{Kr} and I_{Ks} (E-4031 and 293B, respectively). After the I_K reached a practically steady level (control, trace 1), application of E-4031 (3 μmol/l) markedly reduced the amplitude of I_K tail current (trace 2), and further addition of 293B (30 μmol/l) almost completely abolished the I_K tail current (trace 3). Table 2 summarizes densities of I_{Kr} and I_{Ks} in the epicardial, M, and endocardial cells, determined as E-4031- and 293B-sensitive tail currents normalized with reference to C_m. In each cell type, the density of I_{Ks} was significantly smaller than that of I_{Kr}. The density of I_{Kr} was almost equivalent among the three cell types, whereas I_{Ks} density was significantly smaller in M cells compared with that in the epicardial and endocardial cells.

Computer simulations. To understand why EAD developed from the epicardium under the acquired LQTS

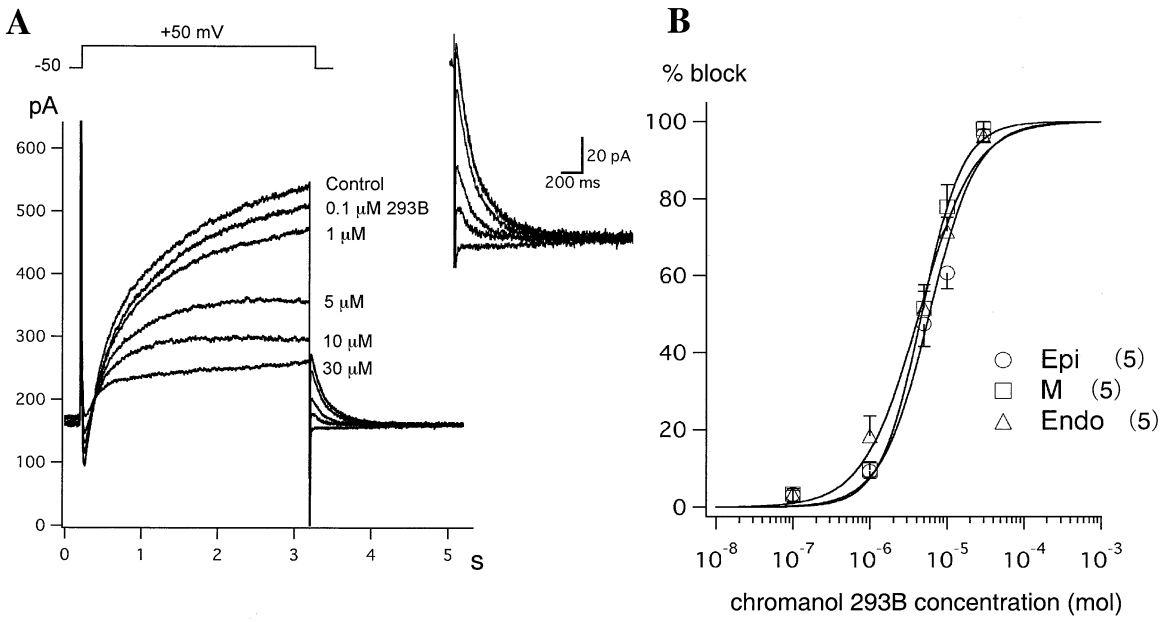

Figure 4. Sensitivity of I_{Ks} in the epicardial (Epi), midmyocardial (M), and endocardial (Endo) cells to inhibition by chromanol 293B. **(A)** Representative superimposed current traces elicited by 3-s depolarizing voltage-clamp steps applied from a holding potential of −50 mV to +50 mV in an epicardial cell, before (control) and during exposure to 293B at a concentration of 0.1, 1, 5, 10, and 30 μmol/l. The I_{Kr} inhibitor E-4031 (3 μmol/l) was present throughout. Tail currents were demonstrated on an expanded scale. **(B)** The percent block of I_{Ks} in the Epi **(open circles)**, M **(open squares)**, and Endo **(open triangles)** cells. The degree of I_{Ks} inhibition was measured as the fraction of the tail current reduced by each concentration of 293B with reference to the control amplitude of the tail current. **Smooth curves** through the data points represent a least-squares fit of a Hill equation: percent block = $100/(1 + (IC_{50}/[293B])^{nH})$, yielding the concentration required for the half-maximal block (IC_{50}) and the Hill coefficient (n_H). pA = pico ($\times 10^{-12}$) Ampere.

condition, we simulated APs of the three cell types using a Luo-Rudy model at a BCL of 2,000 ms. As shown in Figure 6A, the epicardial APD was shorter than the M cells under the control condition (dotted line). However, suppression of both I_{Kr} and I_{Ks} (70% and 80%, respectively) (solid line), simulating the condition of acquired LQTS, developed EAD (arrow) from the epicardial cell but not from M or endocardial cells. Moreover, Figure 6B shows that the reactivation of Ca^{2+} current through the L-type channel ($I_{Ca,L}$) was responsible for the development of epicardial EAD under the acquired LQTS condition. Furthermore, a decrease in I_{to} density changed by G_{to} from 0.5 to 0.05

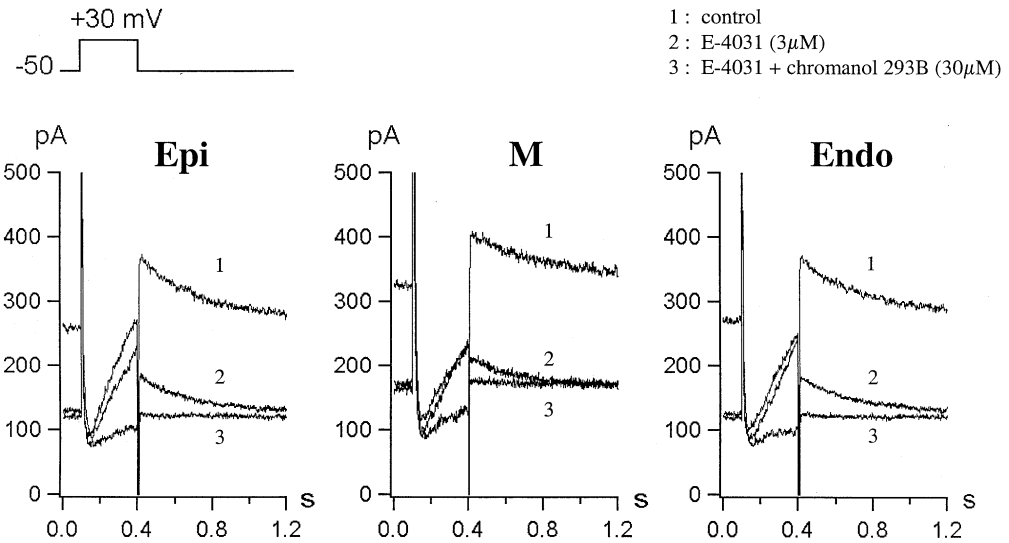

Figure 5. Detection of I_{Kr} and I_{Ks} in the epicardial (Epi), midmyocardial (M), and endocardial (Endo) cells. Depolarizing test pulses (to +30 mV for 300 ms) were repetitively applied (every 2 s) from a holding potential of −50 mV to activate I_K, and membrane currents were recorded from the Epi, M, and Endo cells, before (trace 1), and ~2 min after exposure to 3 μmol/l E-4031 (trace 2), and ~2 min after further addition of 30 μmol/l 293B in conjunction with 3 μmol/l E-4031 (trace 3). pA = pico ($\times 10^{-12}$) Ampere.

Table 2. Transmural Heterogeneity of I_{Ks} and I_{Kr} in Feline Left Ventricle

	Epi (n = 10)	M (n = 9)	Endo (n = 7)
I_{Ks}	0.35 ± 0.26*	0.13 ± 0.09*†	0.30 ± 0.09*
I_{Kr}	1.34 ± 0.51	1.10 ± 0.38	1.17 ± 0.30

*$p < 0.05$ vs. I_{Kr}; †$p < 0.05$ vs. Epi and Endo by analysis of variance with Bonferroni's test. Mean ± SD, (pA/pF). Current densities of I_{Kr} and I_{Ks} measured as E-4031- and chromanol 293B-sensitive tail currents at −50 mV.

Abbreviations as in Table 1.

mS/μF decreased the net charge entry carried by the $I_{Ca,L}$ during the AP, resulted in suppressing EAD as well as abbreviating APD.

DISCUSSION

Genetic and ionic substrates of acquired LQTS. Acquired QT prolongation and TdP arrhythmias usually require multiple risk factors, such as bradycardia, hypokalemia, female gender, and mostly agents with an I_{Kr}-blocking effect. Recent genetic studies suggest some forms of acquired LQTS can be associated with silent mutations in the LQTS-related genes (4), such as *KCNQ1* encoding I_{Ks} (so-called forme fruste type of congenital LQTS) (5–7). Roden (20) hypothesized "reduced repolarization reserve" as a potential mechanism underlying susceptibility to drug-induced LQTS. According to his hypothesis, I_{Ks} dysfunction could be potentially compensated by other K^+ currents, mainly I_{Kr}, thereby the repolarization defect is tolerated, and agents with I_{Kr} block could induce acquired QT prolongation and TdP.

Vos et al. (21–23) suggested a high incidence of EADs and TdP by *d*-sotalol in dogs with chronic complete atrioventricular block as a result of a significant down-regulation of I_{Ks} and I_{Kr}. Moreover, other experimental studies using canine and rabbit wedge showed combined I_{Ks} and I_{Kr} block caused a high incidence of EADs most likely arising from the epicardium (14,15). Burashnikov and Antzelevitch (24) suggested that the abundant I_{Ks} in the epicardium and endocardium compared with the M region under normal conditions contributed to the increase in TDR but protected against development of EADs in the epicardium and endocardium in dogs. Thus, I_{Ks} is critically important for the repolarization reserve in the epicardium and endocardium.

Although I_{Ks} in the feline heart is far smaller than that in other species (25,26), our result from a whole-cell patch-clamp study suggested that a 10-μmol/l 293B used in the wedge preparation reduced about 70% of I_{Ks} in the three cell types, which is consistent with degree of I_{Ks} blockade caused by a silent mutation or common polymorphism in human *KCNQ1* gene (6,7). We also showed that I_{Kr} block with E-4031 in control conditions prolonged the QT interval but did not increase TDR and developed neither EADs nor TdP. However, combined I_{Kr} block with 293B further prolonged the QT interval and inverted T wave, which, in turn, increased TDR and induced EADs and TdP. There-

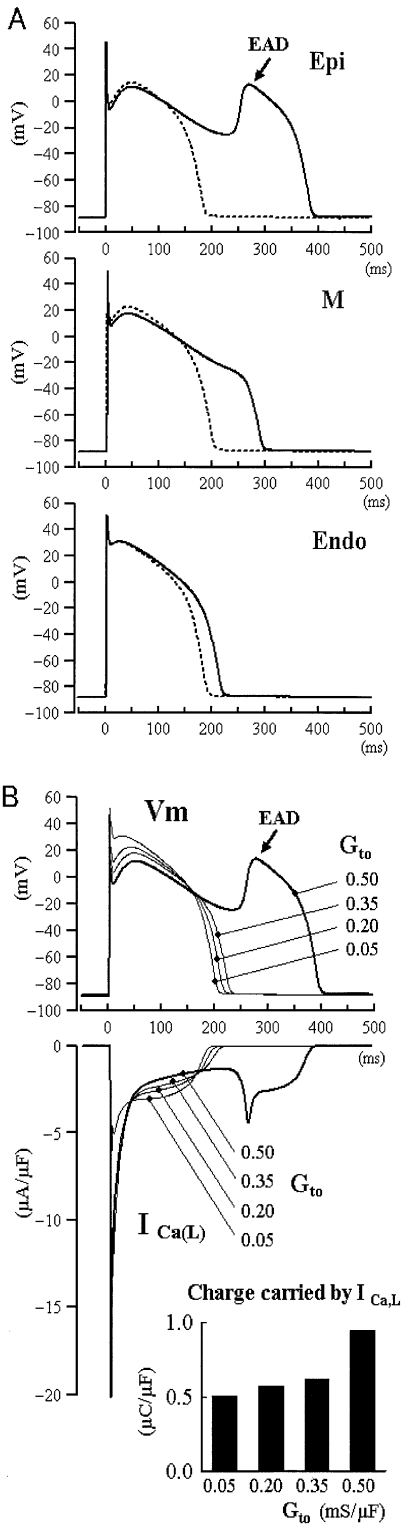

Figure 6. Effect of both I_{Kr} and I_{Ks} suppression on the simulated action potentials from the epicardial (Epi), midmyocardial (M), and endocardial (Endo) cells. **(A)** Superimposed action potentials simulated under baseline condition **(dotted lines)** and after both I_{Kr} and I_{Ks} suppression (70% and 80%, respectively) **(solid lines)**. **(B)** Effect of maximum conductance of I_{to} (G_{to}) on the simulated epicardial action potential (Vm), $I_{Ca,L}$ magnitude, and the net charge entry calculated by integration of the $I_{Ca,L}$ under the condition of both I_{Kr} and I_{Ks} suppression. Basic cycle length = 2,000 ms. EAD = early afterdepolarization.

fore, the feline heart is appropriate for a model of forme fruste LQTS. Our data also suggested that subclinical I_{Ks} dysfunction may become a genetic substrate, and additional I_{Kr} suppression may unmask marked QT prolongation and TdP in acquired form of LQTS.

Role of $I_{Ca,L}$ in increasing TDR and inducing EADs and TdP in acquired LQTS. Several clinical and experimental studies have suggested that EADs and triggered activity were important in the genesis of QT prolongation and TdP in LQTS (8,9,11–15,22–24). Induction of EADs generally requires an initiation or conditioning phase controlled by the sum of membrane currents present at the plateau AP (inward depolarization current and outward repolarization current). January and Riddle (27) suggested that the time- and voltage-dependent $I_{Ca,L}$ within its "window" was important in the induction and block of EADs. Luo and Rudy (28) suggested that EADs resulted from a secondary activation of the $I_{Ca,L}$ during the plateau of AP. However, the mechanism responsible for a high incidence of EADs (especially from the epicardium) and subsequent TdP under conditions of severely eliminated outward K^+ current, mimicking acquired LQTS, has not been mechanistically defined.

Our data indicate that accentuation of $I_{Ca,L}$ during the AP plateau preferentially prolonged APD and triggered EADs in the epicardium. This was based on the effect of verapamil on the epicardium. However, it is still unclear whether a larger $I_{Ca,L}$ in the epicardial cell compared with the M or endocardial cells contributed to the development of EADs. Recently, Bányász et al. (29) reported in their AP voltage clamp experiments that the epicardial cell had a pool of Ca^{2+} channels sufficient for a second activation, whereas the endocardial cells did not. Cordeiro et al. (30) also noted that the presence of spike-and-dome AP waveform in the epicardial cells resulted in a greater magnitude of $I_{Ca,L}$. Moreover, several simulation studies demonstrated a strong coupling between $I_{Ca,L}$ and I_{to} (31,32). Our simulation study also suggested that larger I_{to} in the epicardial cell caused larger $I_{Ca,L}$, developing EADs under the acquired LQTS condition. In the feline left ventricle, it has been reported that I_{to} is larger in the epicardium compared with the endocardium (33). Therefore, larger $I_{Ca,L}$ secondary to I_{to}-mediated spike-and-dome AP configuration in the epicardial cell might be responsible for the high incidence of EADs from the epicardium. This does not necessarily exclude the possible mechanisms of other ionic currents such as I_{NaCa} and Ca^{2+} release from sarcoplasmic reticulum, which may contribute to the prolonged AP as well as to the development of EADs under calcium-loading conditions (34).

Effects of catecholamines and verapamil in acquired LQTS. Treatment of drug-induced TdP begins with immediate withdrawal of any potential drugs and risk factors. Sanguinetti et al. (35) suggested that an increase of heart rate by isoproterenol was an effective therapeutic strategy in patients with acquired LQTS, because beta-adrenergic stimulation with isoproterenol abbreviates repolarization not only by increasing heart rate, but also by directly increasing the magnitude of I_{Ks}. However, our experimental data shows that epinephrine further prolonged APD in the epicardium and induced EADs and TdP probably due to augmentation of $I_{Ca,L}$ in the acquired LQTS condition. Thus, beta-adrenergic stimulation could be arrhythmogenic even in conditions of acquired LQTS when subclinical I_{Ks} dysfunction is present and heart rate is not fully increased.

Cosio et al. (8) used intravenous verapamil to treat three patients with TdP during an atrioventricular block. Shimizu et al. (9) reported that verapamil suppressed spontaneous or epinephrine-induced EADs and TdP in patients with congenital LQTS. Experimentally, Kimura et al. (36) reported that verapamil (2 μmol/l) suppressed cocaine-induced EADs in the myocytes isolated from feline left ventricle. Taken together with the data in the present study, $I_{Ca,L}$ block with verapamil may be a therapeutic choice for TdP in patients with acquired LQTS as well as congenital LQTS.

Study limitations. We assumed the activity recorded from the cut surface of the perfused wedge preparation represented cells within the respective layers of the wall throughout the wedge. Such validation was provided in previous studies that used the wedge preparation (10–15).

Pharmacologic block of I_{Ks} with 293B is not a complete surrogate for *KCNQ1* defect. However, our feline model closely mimicked the degree of I_{Ks} inhibition and pharmacologic features of acquired LQTS. Therefore, we believe these qualitative similarities validate 293B as a surrogate for forme fruste LQTS.

We simulated APs of the three cell types using a Luo-Rudy model, but it does not completely represent feline ventricular APs. However, the phenomenon that EAD frequently developed from the epicardium under the acquired LQTS condition was observed not only in cats but also in dogs and rabbits (14,15); thus, this simulation may support our speculation about the mechanism of this phenomenon.

Finally, the concentration of verapamil mainly used in this study (2.5 μmol/l = 1,250 ng/ml) was considerably higher than a typical clinical dose. However, verapamil was effective in suppressing EADs and decreasing TDR even at the lowest dose used in this study (0.1 μmol/l = 50 ng/ml), which is close to plasma concentration of verapamil after a 5-mg bolus injection (below 200 ng/ml).

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